lifting inhibition. The force-sensing ability of titin kinase was demonstrated in AFM experiments and simulations [Puchner, et al., 2008, PNAS:105, 13385], which showed indeed that mechanical forces can remove the autoinhibitory tail of titin kinase. We report here steered molecular dynamics simulations (SMD) of the very recently resolved crystal structure of twitchin kinase, containing the kinase region and flanking fibronectin and immunoglobulin domains, that show a variant mechanism. Despite the significant structural and sequence similarity to titin kinase, the autoinhibitory tail of twitchin kinase remains in place upon stretching, while the N-terminal lobe of the kinase unfolds. The SMD simulations also show that the detachment and stretching of the linker between fibronectin and kinase regions, and the partial extension of the autoinhibitory tail, are the primary force-response. We postulate that this stretched state, where all structural elements are still intact, may represent the physiologically active state.

## Excitation Contraction Coupling I

**1833-Pos Board B603**

**FRET Reveals Substantial Reorientation of the Cytoplasmic Interface of the Skeletal Muscle DHPR in the Presence of RyR1**

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In skeletal muscle, the dihydropyridine receptor (DHPR) in the t-tubular membrane serves as Ca2+channel and as voltage sensor for excitation-contraction (EC) coupling, triggering Ca2+release via a physical/conformational coupling to the type 1 ryanodine receptor (RyR1) in the sarcoplasmic reticulum (SR) membrane. The particulars of the structural and functional links between these two proteins are widely unknown. The putative intracellular portions of the DHPR a1S subunit, the N-terminus, C-terminus, and the loops connecting the four homologous repeats (I-IV), play important roles in the communication with the RyR1. Examples are the β-subunit recruiting function of the I-II loop, the bi-directional signaling function of the II-III loop with the RyR1 during EC-coupling, the influence of the III-IV loop on RyR1 mediated Ca2+delivery, and the a1S C-terminus. These channel parts are believed to either directly or indirectly interact with the RyR1, and the close spatial proximity between the two channels at t-tubule/SR 'junctions' constitutes the structural prerequisite for this linkage. The present work provides for the first time a structural insight into the arrangement of the crucial molecular components of the DHPR-RyR1 interaction, by using measurements of fluorescence resonance energy transfer (FRET), conducted within the cellular environment of living myotubes. Upon expression, the degree of FRET was determined for different combinations of labeled cytoplasmic a1S domains, using a sensitized emission FRET variant. Confocal fluorescence microscopy was applied to check for correct expression and function of the constructs upon expression in dyspedic (RyR1 null) and dysgenic (a1S null) myotubes. The presence of RyR1 significantly altered the intramolecular energy transfer for almost every double tagged a1S construct. These measurements reveal that virtually the complete cytoplasmic a1S architecture is significantly rearranged by the presence of the RyR1.

**1834-Pos Board B604**

**19-Residue Peptide from C-Terminal Tail of DHPR β1A Subunit Potentiates Voltage-Dependent Calcium Release in Adult Skeletal Muscle Fibers**

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The skeletal muscle isoforms of the membrane-spanning $\alpha_{1s}$ subunit and the cytoplasmic $\beta_{1a}$ subunit are essential during EC coupling. Recent evidence suggests that the activating effect of the full-length $\beta_{1a}$ subunit on isolated ryanodine receptor (RyR1) $Ca^{2+}$ channels in lipid bilayer can be reproduced by a peptide ($\beta_{1a}$ 490-524) corresponding to the 35-residue C-terminal tail of the $\beta_{1a}$ subunit and also confirmed a high-affinity interaction between the C-terminal tail of the $\beta_{1a}$ and RyR1. We now tested the hypothesis that a 19 amino acid residue peptide ($\beta_{1a}$ 490-508) may be sufficient to reproduce activating effects already observed for $\beta_{1a}$ 490-524 as well as that of the full-length peptide. The hypothesis is based on existing results using overlapping peptides tested on isolated RyR1 in phospholipid bilayer (1). Here we examined the effects of $\beta_{1a}$ 490-508 on $Ca^{2+}$ release during whole cell voltage-clamp depolarization of adult mouse FDB muscle fibers. 25 nM or 100 nM of $\beta_{1a}$ 490-508 peptide in a patch pipette caused a 25% increment in the SR $Ca^{2+}$ release flux in single voltage clamped muscle fibers but with no significant shift in the voltage dependence of the maximum peak $Ca^{2+}$ release flux. Considerably less activating effect was observed using 400 nM peptide. A scrambled form of the 19-residue peptide (100nM) was used as a negative control for the wild-type peptide and produced a negligible effect on the peak amplitude of $Ca^{2+}$ release flux. Taken together, we have shown that the $\beta_{1a}$ 490-508 peptide contains molecular components sufficient to modulate EC coupling between DHPR and RyR1 in adult functioning muscle fibers. Supported by R01-AR055099 and T32-AR007592.

1. Rebbeck, R.T. et. al. March (2011) Biophysical Society Abstract 3195-Pos/B300.

**1835-Pos Board B605**

**Role of STIM1 in Skeletal Excitation-Contraction Coupling**

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STIM1, a $Ca^{2+}$-sensing protein on ER/SR membrane, mediates a store-operated $Ca^{2+}$-entry (SOCE) by activating Orai1 $Ca^{2+}$-entry channel on plasma membrane. E136X mutant of STIM1, truncated STIM1 missing binding abilities to Orai1, has been found in patients with immunodeficiency accompanying muscular hypotonia. To identify causes of the muscular hypotonia, E136X was expressed in mouse skeletal myotubes and dominant-negative effects of E136X were examined. Myotubes expressing E136X showed increases in both KCl (a membrane depolarizer resulting excitation-contraction coupling) and caffeine (a direct RyR1 agonist) responses. On the other hand, SOCE, resting cytosolic $Ca^{2+}$ level, SR $Ca^{2+}$ level were not significantly changed by the expression of E136X. In addition, E136X did not interfere with puncta formations by endogenous STIM1 and Orai1. These data mean that muscular hypotonia found in patients with E136X is due to changes in excitation-contraction coupling. Additionally, we suggest that C-terminus of STIM1 that is missing in E136X participates in the regulation of skeletal EC coupling.

**1836-Pos Board B606**

**Direct Quantification of Calsequestrin-Dependent Buffering in the Calcium Store of Skeletal Muscle**

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In mouse FDB cells releasing $Ca^{2+}$ under patch clamp we measured luminal $[Ca^{2+}]_{SR}$ with the novel sensor D4cpv-calsequestrin (Sztretye *et al.* JGP 2011a) in parallel with SR $Ca^{2+}$ release flux (derived from the fluorescence of cytosolic X-rhod 1). These simultaneous measurements allowed the dynamic monitoring of $Ca^{2+}$ buffering power of the SR ($BP \equiv \Delta[Ca]_{total,\ SR} / \Delta[Ca^{2+}]_{SR}$). *BP* started at 180 (SEM 30, 27 cells) and decreased to 89 (SEM 14) as the SR lost $Ca^{2+}$ upon SR-depleting membrane depolarization (400 ms, +30 mV). The stage of high *BP* is also characterized by a "hump" in the release flux waveform. Recovery of SR $Ca^{2+}$ content after the end of depolarization proceeded at the lower *BP* value (a mismatch that we call "buffer hysteresis"). *BP* regained slowly its initial value, as demonstrated by persistent low power and absence of hump in the second depolarizing pulse of pairs separated by 600 ms. Full restoration of hump and *BP* was observed after 2 min rest. In calsequestrin1-null cells *BP* was a constant 40 during the pulse (3.6, 29 cells), there was neither buffer hysteresis nor flux hump. Therefore, calsequestrin 1 is the time-dependent, hysteretic buffer, and contributes approximately 75% of the SR buffering power. Total releasable $Ca^{2+}$, however, only decayed by 30% in CSQ-null cells, a paradox explained by a more thorough depletion in the null (Sztretye *et al*. JGP 2011b). The time dependence and hysteresis of the calsequestrin contribution to $Ca^{2+}$ buffering is consistent with aggregation changes proposed to accompany $Ca^{2+}$ binding (e.g. Park *et al*. JBC 2004). In this view, the hysteresis reflects the time required for the $Ca^{2+}$ dissociation-induced structural changes in calsequestrin to reverse upon $Ca^{2+}$ reuptake into the SR.
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**1837-Pos Board B607**

**Two-Edged Sword: The $Ca^{2+}$ Biosensor D4cpv-Calsequestrin Restores Functionality to Calsequestrin-Null Muscle**

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SR $Ca^{2+}$ buffering power, *BP*, decreases during $Ca^{2+}$-depleting depolarizations of mouse skeletal muscle. During $Ca^{2+}$ release the stage of high *BP* is characterized by a "hump" in the release flux waveform. After the depolarization *BP* returns slowly to its initial value, as demonstrated by the absence of a hump in the flux induced by the second pulse of pairs separated by 600 ms. These time-dependent features were described as "buffer hysteresis" and shown to be contributed by calsequestrin in the Sztretye *et al.*, companion poster. SR release flux and *BP* were measured in calsequestrin 1-null cells expressing the biosensor D4cpv-calsequestrin. Null cells had lower *BP* and *generally* lacked the hump in the flux. In some regions of these cells, however, [biosensor] reached very